IMPROVEMENTS IN OIL-SECTIONING WITH COLLODION.

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Everyone who works much with any method must almost inevitably find out certain modifications which make the method more efficient and more easily applied. If one has also to adapt methods to the needs of laboratory students, modifications become almost imperative, and frequently the necessary improvements naturally grow up in aiding students to meet their special requirements.

In the perfection of the collodion method of sectioning the two greatest advances were made: (1) in learning to handle the sections with paper and (2) in clarifying the tissue and the surrounding collodion mass with an essential oil. Weigert ('85) seems to have been the one to first publish the paper-method of handling sections, and so far as I can judge, Bumpus ('92) first published a practicable method of clarifying the imbedded tissue and mass in oil, and of using oil to float the sections on the knife as they were cut. In the "oil method" of Bumpus the object must be stained *in toto* and, after transferal to the slide and most of the oil absorbed, mounted immediately in balsam. *In toto* staining is not by any means applicable to all tissues and for all work, especially morphological work where large organs or entire animals are sectioned. To obviate the defects in Bumpus' method Dr. P. A. Fish made two important improvements: (1) He mixed with the essential oil (oil of white thyme) one-third its bulk of castor oil, thus avoiding the rapid drying, and (2) he fastened the sections to the slide after the oil had been well removed with absorbent paper by adding a small amount of a mixture of ether and alcohol. The ether and alcohol were allowed to evaporate until the surface began to look glazed, then the slide bearing the sections was placed in ninety-five per cent. alcohol to remove the oil, then passed through seventy per cent. and

thirty-five per cent. alcohol to water, if a watery stain like hematoxlin was to be used. Finally after the stain was washed away with water, the sections were dehydrated by again passing them through thirty-five per cent., seventy per cent. and ninety-five per cent. alcohol and cleared and mounted in balsam, thus rendering it possible to use any stain desired after the sections were cut. This method as perfected by Dr. Fish is truly admirable.

The improvements which have been evolved by my own experience are mostly in the direction of simplification and cheapening. For the sake of those who may not be familiar with the collodion method there will be given in very brief form the entire method, pointing out at the end the special improvements which it is the purpose of this paper to put on record:

1. *Fixing and Hardening.*—The tissue, organ, embryo or animal of which sections are desired is fixed, and hardened by any of the standard or special methods.

2. *Dehydration.*—The tissue is thoroughly dehydrated by using one or more changes of plentiful ninety-five per cent. alcohol or by the use of absolute alcohol. It is better not to dehydrate more than twenty-four hours. By changing the alcohol three or four times, two or three hours is sufficient for small pieces of material, and five or six hours will suffice for the larger objects.

3. The tissue is thoroughly saturated with a mixture of equal parts of sulphuric ether and ninety-five per cent. or stronger alcohol. This requires two to five hours for small objects. Over night does no harm. The ether and alcohol complete the dehydration and prepare the tissue more perfectly than alcohol alone for the infiltration with collodion.

4. *Infiltration with Thin Collodion.*—The ether-alcohol is poured off, and a mixture of thin collodion (ether and ninety-five per cent. alcohol, equal parts, 100 cc.; soluble cotton one and one-half grams). Two or three hours will suffice for objects two or three millimeters in thickness. A stay of one or more days does no harm. The larger the object the more time is needed.

5. *Infiltration with Thick Collodion.*—The thin collodion is poured off and thick collodion added (ether and alcohol, equal

parts, 100 cc.; soluble cotton*, six grams). For very small objects four or five hours will suffice to infiltrate, but for larger objects a longer time is necessary. The tissue does not seem to be injured at all in the thick collodion, and a stay in it during a day or even of a week or more is more certain to insure a perfect infiltration.

6. *Imbedding and Hardening the Mass.*—The tissue may be imbedded in a paper box such as is used for paraffin imbedding, or in any of the other boxes devised for paraffin. It is better, if paper is used, to put a very small amount of oil on the paper to prevent the collodion from sticking to it. Vaseline spread over lightly and then all removed so far as possible with a cloth or with lens paper gives the right surface. For small objects it is more convenient to imbed immediately on a holder that may be clamped into the microtome. Cylinders or blocks of glass, vulcanite, wood and cork have all been recommended and used. A cork of the proper size is most convenient and for many purposes answers well. Some collodion is put on the end of the cork and a pin put near one edge. The tissue is transferred from the thick collodion to the cork and leaned against the pin. Drops of the thick collodion are then poured on the tissue and by moving the cork properly, the thick, viscid mass

*The substance used in preparing collodion goes by various names, soluble cotton or collodion cotton is perhaps best. This is cellulose nitrate, and consists of a mixture of cellulose tetranitrate $C_{12}\ H_{16}\ (NO_3)4\ O_6$, and cellulose pentanitrate, $C_{12}\ H_{15}\ (NO_3)5\ O_5$. Besides the names soluble and collodion cotton, it is called gun cotton and pyroxylin. Pyroxylin is the more general term and includes several of the cellulose nitrates. Celloidin is a patent preparation of pyroxylin, more expensive than soluble cotton, but in no way superior to it.

Soluble cotton should be kept in the dark to avoid decomposition. After it is in solution this decomposition is not so liable to occur. The decomposition of the dry cotton gives rise to nitrous acid, and hence it is best to keep it in a box loosely covered so that the nitrous acid may escape.

Cellulose nitrate is explosive under concussion and when heated to 150° centigrade. In the air, the loose soluble cotton burns without explosion. It is said not to injure the hand if held upon it during ignition and that it does not fire gun-powder if burned upon it. So far as known to the writer, no accident has ever occurred from the use of soluble cotton for microscopical purposes. I wish to express my thanks to Professor W. R. Orndorff, organic chemist in Cornell University for the above information.

may be made to surround and envelop the tissue. Drops of collodion are added at short intervals until the tissue is well surrounded, and then as soon as a slight film hardens on the surface, the cork bearing the tissue is inverted in a wide-mouth vial

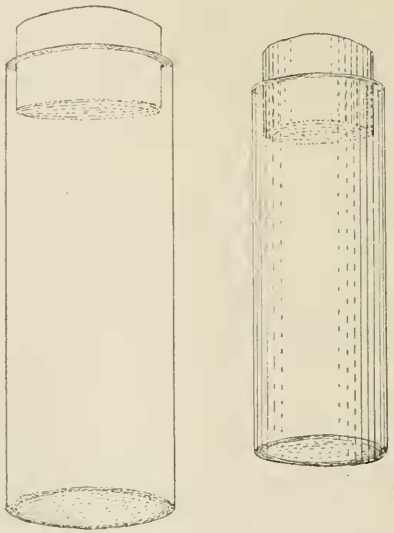

Fig. 1. Wide-mouth vials for the purposes of Histology and Embryology. These represent the two vials natural size that have been found most useful. They are kept in blocks with holes of the proper size. They are especially useful for objects treated by the collodion method as described in this paper.

of considerably larger diameter than the cork (Fig. 1). The vial should contain sufficient chloroform to float the cork. The vial is then tightly corked. In imbedding somewhat larger objects on the end of a cork or other holder it is frequently advantageous to wind oiled paper around the holder or cork, tie it tightly and have the projecting hollow cylinder sufficiently long to receive the object. The tissue is then put into the cylinder and sufficient collodion added to completely immerse it. As soon as a film has formed over the exposed end, the cork may be inverted and immersed in chloroform as described above.

7. *Hardening and Clarifying the Collodion.*—After a few hours

the collodion is hardened by the chloroform. If it acts long enough the imbedding mass is rendered entirely transparent if no water is present. Whenever the collodion is hard, whether it is clear or not, the chloroform is poured off and the *clarifier* added (the clarifier is made by mixing thoroughly xylene* three parts with castor oil one part by volume.) In a few hours the imbedded mass will become as transparent as glass and the tissue will seem to have nothing around it. Sometimes the collodion remains white and opaque for a considerable time. So far as the writer has been able to judge, this is due to moisture. If one breathes on the mass too much while imbedding, or if it is very damp in the room, the opacity may result. Sometimes, in objects of considerable size, this may remain for a week. This is the exception, however, and if the mass seems sufficiently hard and tough the cutting may proceed even if the clarification is incomplete.

8. *Cutting the Sections.*—For collodion sectioning a long drawing cut is necessary in order to obtain thin, perfect sections. The object is therefore put in the jaws of the microtome at the right level and the knife arranged so that half or more of the blade of the knife is used in cutting the section. It is advantageous also to have the object placed with its long diameter parallel with the edge of the knife. The surrounding collodion mass should be cut away so that there is not more than a thickness of about two millimeters all around the tissue. This is to render the diameter of the end to be cut as small as possible. The smaller the object the thinner can the sections be made. With an object two to three millimeters thick and not over five millimeters wide and a good sharp knife, sections 5μ to 6μ can be cut without difficulty. When knife and tissue are properly arranged the tissue is well wet and the knife flooded with the clarifier. (For wetting object and knife during the sectioning a mixture of xylene four parts and castor oil one part is rather better than the ordinary clarifier as it is somewhat more fluid).

*The hydrocarbon xylene (C_8 H_{10}) is called xylol in German. In English, members of the hydrocarbon series have the termination "ene" while members of the alcohol series terminate in "ol."

Make the sections with a steady motion of the knife. Then draw the section up toward the back of the knife with an artist's brush and make the next section. Arrange the sections in serial order on the knife blade till enough are cut to fill the area that the cover-glass will cover.

9. *Transferring the Sections to the Slide.*—If the clarifier has evaporated so as to leave the sections somewhat dry, on the knife, add a small amount. Take a piece of thin absorbent, close-meshed paper* about twice the size of a slide and place it directly upon the sections. Press the paper down evenly all around and then pull the paper off the edge of the knife. The sections will adhere to the paper. Place the paper, sections down, on a slide, taking care that the sections are in the desired position on the slide. Use some ordinary lens-paper or any absorbent paper and press it down gently upon the transfer paper. This will absorb the oil, and then the transfer paper may be lifted from the slide. The sections will remain on the slide.

10. *Fastening the Sections to the Slide.*—Drop just enough ether-alcohol (equal parts of sulphuric ether and ninety-five per cent. alcohol) on the sections to moisten them. This will melt the collodion and fasten the sections to the slide. Allow the slide to remain in the air till the surface begins to look slightly dull or glazed.

11. *Removing the Oil from the Sections.*—As soon as the ether-alcohol has evaporated sufficiently to leave the surface dull, place the slide in a jar of ordinary commercial benzin. It may be left here a day or more without injury to the sections, but if moved around in the jar the oil will be removed in three to five minutes. From the benzin transfer to a jar of ninety-five per cent. alcohol to wash away the benzin. One may use alcohol in the beginning, but it dissolves the oil far less rapidly than the

*Various forms of paper have been used to handle the collodion sections. It should be moderately strong, fine meshed and not liable to shed lint, and fairly absorbent. One of the first and most successful papers recommended is "closet or toilet paper." Cigarette paper is also excellent. In my own work the silky Japanese paper called "Usago" paper has been found almost perfect for the purpose. Ordinary lens paper or thin blotting paper for absorbing the oil is used with it.

benzin. The slide may remain in the alcohol half a day or more if one wishes, but a stay of five minutes or a thorough rinsing of half a minute or so by moving the slide around in the alcohol will suffice.

12. *Staining the Sections.*—(A), *With an alcoholic stain.* If an alcoholic stain containing fifty per cent. or more alcohol (for example hydrochloric acid carmine in seventy per cent. alcohol) is used, the slide may be removed from the ninety-five per cent. alcohol, drained somewhat and then the stain poured upon the sections or preferably the slide immersed in a jar of the stain. The stain is finally washed away with sixty-seven per cent. or stronger alcohol, the sections dehydrated in ninety-five per cent. alcohol, cleared and mounted in balsam.

(B) *With an aqueous stain like hematoxylin, etc.*—If an aqueous stain is to be used the sections must first be rinsed with water. In the past the plan for changing sections from ninety-five per cent. alcohol to water for example, has been to run them down gradually, using seventy-five, fifty and thirty-five per cent. alcohol successively. Each percentage may vary, but the principle of a gradual passing from strong alcohol to water was advocated. On the other hand, I have found that the safest method is to plunge the slide directly into water from the ninety-five per cent. alcohol. The diffusion currents are almost or quite avoided in this way. There is no time for the alcohol and water to mix, the alcohol is washed away almost instantly by the flood of water. So in dehydrating after the use of watery stains, the slide is plunged quickly into a jar of ninety-five per cent. alcohol. The diffusion currents are avoided in the same way, for the water is removed by the flood of alcohol. This plan has been submitted to the severe test of laboratory work and has proved itself perfectly satisfactory.

In staining with a watery stain then, the slide bearing the sections is transferred from the ninety-five per cent. alcohol and plunged into a jar of water, and either allowed to remain a few minutes or moved around in the water a moment. Then it is placed horizontally and some of the stain placed on the sections,

or preferably it is immersed in a jar of the stain; in case of immersion, however, the slide should stand vertically or nearly so, then any particles of dust, etc., in the stain will settle to the bottom of the vessel and not settle on the sections. When the sections are stained they are thoroughly washed with water either by the use of a pipette or by immersing in a jar of water. They may then be counterstained with some general dye like eosin or picric acid or mounted with but the one stain.

13. *Mounting in Balsam.*—After the sections are stained they must be dehydrated and cleared before mounting in balsam. For the dehydration the slide is plunged into a jar of ninety-five per cent. alcohol. This removes the water so rapidly that the injurious diffusion currents, which loosen or tear the sections, are avoided. For clearing after the dehydration the slide is drained of alcohol and put down flat and the clearer poured on, or the whole slide is immersed in a jar of clearer (Carbol-turpentine is a good clearer,—Crystals of carbolic acid melted, two parts, turpentine three parts; or carbol-xylene clearer,—melted crystals of carbolic acid two parts, xylene three parts). Clearing usually is sufficient in a few minutes, a stay of an hour or even over night does not injure most sections.

In mounting in balsam the clearer is drained away by standing the slide nearly vertically on some blotting paper or by using the waste bowl and standing it up in the little funnel. (Fig. 2.) Then the balsam is put on the sections or spread on the cover-glass and that placed over the sections.

The improvements here advocated are: (1) The use of xylene and castor oil, the xylene taking the place of the essential oils heretofore recommended for "oil-sectioning." This is much cheaper and in every way as good, perhaps better; it has also the advantage of being nearly odorless. Imbedded tissues have been preserved in the mixture nearly six months without deterioration.

(2) The term *clarifier* is proposed to avoid using *clearer*, which can then be restricted to its original use, viz.: to displace the alcohol from sections and render them transparent before

adding balsam, the clearer being always quite freely miscible with both alcohol and balsam.

(3) In the avoidance of diffusion currents in changing sections fastened to a slide or cover from liquids of greatly different densities, as from alcohol to water, by plunging the slide directly into the new liquid, the great flood of the new liquid serving to remove the previous liquid so rapidly that there is no chance for the destructive diffusion currents which tend to tear the sections in pieces or loosen them from the slide.

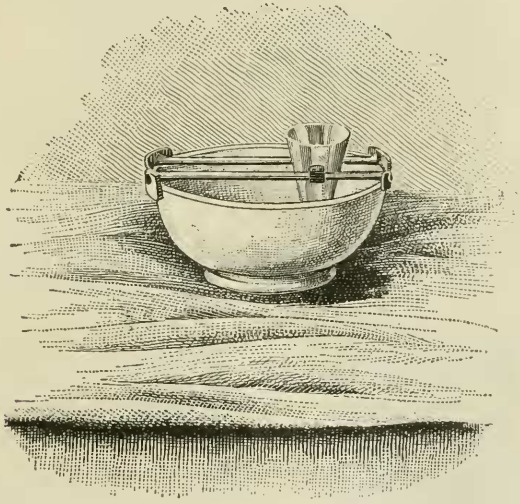

Fig. 2. WASTE BOWL FOR HISTOLOGICAL WORK.—(From the Reference Hand-Book of the Medical Sciences, Supplement.) The glass rods are for resting the slide horizontially and the funnel for draining them.

REFERENCES.

For articles and abstracts or references to articles on the collodion method one can profitably consult the Journal of the Royal Microscopical Society, the Zeitschrift für wissenschaftliche Mikroskopie and the Proceedings of the American Microscopical Society. Special references for the preceding article are:

'92. Bumpus, H. C. A new method of using collodion for serial section cutting. American Naturalist, Vol. xxvi., 1892,

pp. 80–81. In this paper is given the method of sectioning by the use of thyme oil for clarification and for floating the sections on the knife. For Bumpus' "oil-method," see also Journal Royal Microscopical Society, '92, p. 438 and Dr. Fish's papers below.

'93. Fish, P.A,—Recent Histological Formulæ. Reference Handbook of the Medical Sciences, supplement, 1893, pp. 434–436.

'93. Fish, P. A.—A new clearer for collodionized objects. Proceedings American Microscopical Society, vol. xv., 1893, pp. 86–89. It was in this paper that Dr. Fish showed the advantage of adding a fixed oil to the clarifier to be used for "oil-sectioning," and also that sections made by this method could be fastened to the slide and stained as desired.

'91. Gage, S. H.—Albumenizing the slide for the more certain fixation of serial collodion sections. Proceedings American Microscopical Society, vol. xiii., 1891, pp. 82–83. It is shown in this paper that if the sections are to be subjected to long-continued manipulation they may be more surely fixed to the slide by at first albumenizing the slide with egg albumen, one to 200 of water and allowing the albumen to dry on the slide.

'93. Lee, A. B.—The Microtomist's Vade Mecum, 3d Ed., London and Philadelphia, 1893. In this work is given an excellent account of the collodion and celloidin methods and the history of the two methods, with modifications.

'85. Weigert, C.—Ueber Schnittserien von Celloidinpräparaten des Centralnervensystems zum Zwecke der Markscheidenfärbung. Zeit. f. wiss. Mikr., Bd. 2, 1885, pp. 490–495.

In this article is described a method of using paper for the handling of celloidin sections. On p. 491 he says, "closet paper" had been used for a considerable time for this purpose in the Pathological Institute of Heidelberg.

'83. Viallanes.—Rech. sur l'hist. et le dév. des Insects. 1883. Viallanes recommends in this work the use of chloroform for the hardening of the collodion.